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DEVELOPMENT OF UV SPECTROPHOTOMETRIC FIRST ORDER DERIVATIVE METHOD FOR THE SIMULTANEOUS ESTIMATION OF RITONAVIR AND LOPINAVIR IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise analytical method has been developed for the simultaneous estimation of ritonavir and lopinavir in pure bulk drug and in combined tablet dosage form by UV spectrophotometry by first order derivative method. The standard solutions of ritonavir and lopinavir were prepared in acetonitrile followed by further required with the same solvent. The solution containing ritonavir and lopinavir (20µg/mL and 80µg/mL) were scanned between 400 nm to 200 nm and from the overlain first order derivative graph it appeared that ritonavir showed zero crossing at 278.10 nm while lopinavir showed zero crossing at 246.70 nm. At zero crossing point of ritonavir (278.10 nm), lopinavir showed a measurable derivative absorbance where as at the zero crossing point of lopinavir (246.70 nm), ritonavir showed appreciable derivative absorbance value. Thus both the drugs do not interfere in the quantitation of one another. Calibration graphs showed linearity at the concentration ranges from 5-30 µg/ ml by ritonavir and from 20-120 µg/ ml by lopinavir. Corresponding regression equations of both the drugs were used for the determining their concentration. In the bulk drugs, ritonavir and lopinavir were estimated as 99.54% and 100.07% respectively whereas in the marketed tablets ritonavir was found as 98.17 % and lopinavir as 101.6 %. The results of analysis have been validated as per ICH guidelines and were found to be satisfactory. Hence, present study gives excellent method for the determination of both the drugs in combined tablet formulation.

INTRODUCTION: Ritonavir (RIT) and lopinavir (LOP) both are antiretroviral drugs specifically belongs to protease inhibitors class**.** Literature survey reveals some methods for their determination by HPLC [1-4] or spectrophotometry [5-6] either alone or in combination, but no method was found for the selected combined dosage form by first order derivative spectrophotometry. For the routine quantitative estimation of these drugs, a simple, accurate and sensitive method was required. Hence the proposed method was developed.

MATERIAL AND METHODS:

Materials: Shimadzu 1601UV-visible spectrophotometer with a matched pair of 10 mm quartz cells was used. RIT and LOP pure drugs (Cipla Ltd. Goa and Patalganga, INDIA), acetonitrile (LOBA, India Ltd) were used in the present study. The commercially available tablets containing a combination of RIT-50mg and LOP -200 mg were procured from pharmacy.

Methods:

Standard Stock Solution: The stock solutions having 1mg/mL solutions of RIT and LOP were prepared separately by dissolving an accurately weighed quantity of drugs in acetonitrile.

Mixed Standard Solutions: Aliquots of both the stock solutions were mixed and diluted using acetonitrile so as to get the mixed concentrations of RIT and LOP as 5:20, 10:40, 20:80, 15:60 and 25:100 and 30:120 µg/mL, respectively.

Selection of Scanning Range and Sampling Wavelengths: The standard solutions of RIT and LOP were diluted with acetonitrile individually to get the concentration of 20 and 80 µg/mL, respectively and were scanned in the UV range 400-200 nm. The spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 0.1 nm. The two spectra were overlain (**Figure 1**). It appeared that RIT showed zero crossing at 278.10 nm while LOP showed zero crossing at 246.70 nm. At zero crossing point of RIT (278.10 nm), LOP showed a measurable $dA/d\lambda$ where as at the zero crossing point of LOP (246.70 nm), RIT showed appreciable $dA/d\lambda$. Hence, the wavelengths 278.10 nm and 246.70 nm were selected as analytical wavelengths for determination of RIT and LOP, respectively.

Analytical concentration range and plotting of calibration curves: For the linearity study, aliquots of the drug solutions were further diluted with acetonitrile to get the final working standards of concentration range as RIT as 5-40 µg/mL and LOP as 20-120 µg/mL respectively. The first derivative spectra was taken and the derivative absorbance at 246.70 nm and 278.10 nm for RIT and LOP were measured, respectively. The calibration graphs of both the drugs were plotted at 246.70 nm and 278.10 nm (Figure-2 and Figure-3). The following regression equations for both the drugs were used for the quantitative estimation of samples.

RIT-A = (-0.00019)+(-0.00067)xC, r= -0.99918 .....(Eq. 1)

LOP- A = 0.0 + (-0.000025) x C, r = -1 ..... (Eq. 2)

Where, α = Intercept; β= Slope, C= concentration in µg/mL, A= absorbance of the first order derivative curves at 246.70 nm and 278.10 nm for RIT and LOP respectively. r = correlation coefficient.

Estimation of drugs in Standard Laboratory Mixture by proposed method: The mixed standard solutions of RIT and LOP (20:80 µg/mL) were scanned at analytical wavelengths and their derivative absorbance were noted. The concentrations of both the drugs in the mixed standard solution were obtained from the regression equations of RIT and LOP. The results are depicted in **Table 1**.

Application of proposed method for the estimation of RIT and LOP in tablets: Tablets containing RIT (50 mg) and LOP (200 mg) were weighed and finely powdered. A quantity of powder equivalent to RIT (50 mg) was accurately weighed and transferred to volumetric flask and was dissolved in acetonitrile. The solution was then filtered through Whatman filter paper No.1 and the volume was made up to 50 mL. Aliquot of the solution was diluted with acetonitrile to get the working standards of RIT as 20µg/mL (~LOP as 80µg/mL). The sample solution was scanned for the absorbance at 246.70 nm and 278.10 nm. The concentration of both the drugs in the tablet solution was determined by using the regression equation. The results are shown in Table 1.

Recovery Studies: To the preanalyzed tablet powder solution, pure drugs of RIT (10 mg) and LOP (20 mg) were added.The mixture was shaken thoroughly and diluted upto the mark with acetonitrile and filtered through Whatman filter paper No.1. Aliquot portions of the resultant solution was appropriately diluted with acetonitrile to get concentrations within the range of mixed standards. The derivative absorbance of the solutions was measured at the analytical wavelengths.

The concentration of the two drugs in the mixed standard solution was obtained from the regression equations of RIT and LOP, respectively.The weight of RIT and LOP contributed by tablet powder calculated earlier, was deducted from total RIT and LOP estimated. The remaining amount of drug was assumed to be recovered from that was added. The results of recovery studies on the marketed prepartion are shown in Table 1.

Validation Parameters: Study of some validation parameters like accuracy (recovery studies), precision(S.D), specificity and ruggedness were carried out as per ICH guidelines and the results are shown in **Table 1 and 2**.

RESULTS AND DISCUSSION:

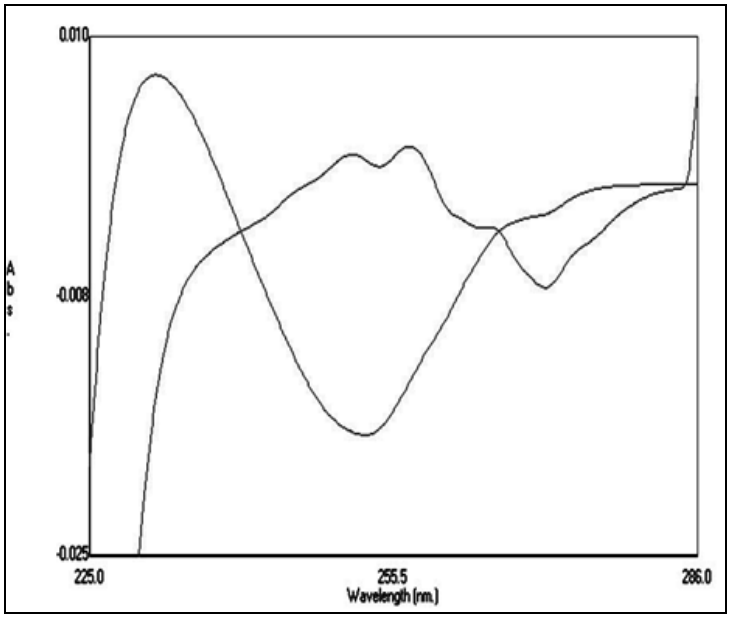

FIGURE 1: FIRST ORDER DERIVATIVE OVERLAIN SPECTRA OF RIT AND LOP

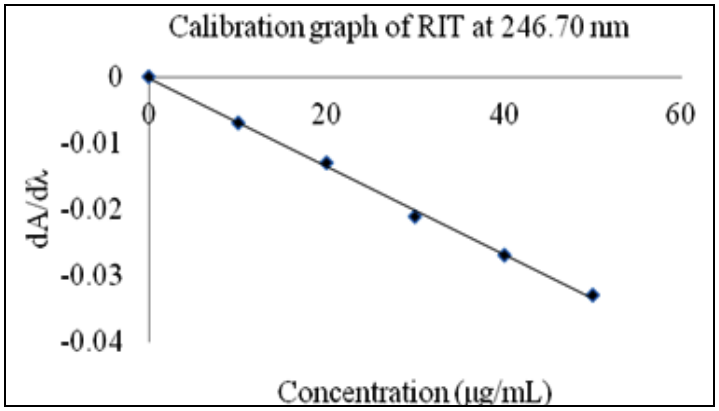

FIGURE 2: CALIBRATION CURVE OF RIT AT 246.70 nm

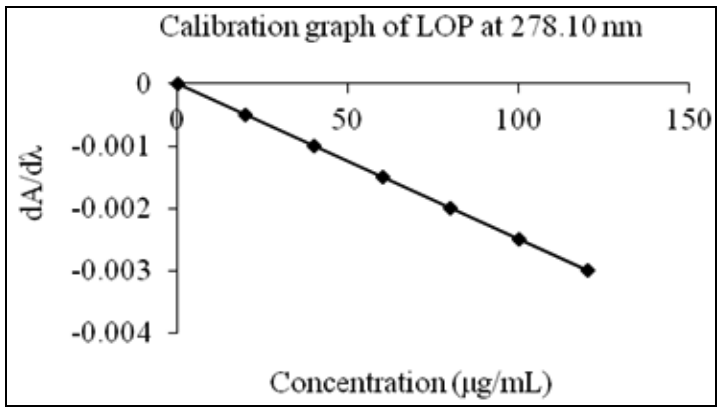

FIGURE 3: CALIBRATION CURVE OF LOP at 278.10 nm

TABLE 1: ANALYSIS OF STANDARD LABORATORY MIXTURE, MARKETED TABLETS AND RECOVERY STUDIES FOR RIT AND LOP BY PROPOSED METHOD

	Drug	% Mean	±SD	SE	CV
Standard laboratory mixture	RIT	99.54	0.42	0.188	0.0042
	LOP	100.07	0.14	0.063	0.0014
Tablets	RIT	98.17	0.43	0.19	0.0043
	LOP	101.6	0.55	0.24	0.0054
Recovery studies	RIT	99.05	0.17	0.076	0.0017
	LOP	99.03	0.21	0.094	0.0021

TABLE 2: RESULTS OF SPECIFICITY AND RUGGEDNESS

Specificity parameters		
Sample	% label claim	
	RIT	LOP
Normal	95.15	93.31
Alkali	24.8	37.5
Acid	45.1	32.4
Oxide	40.5	43.6
Ruggedness parameters: i) Different analyst		
Analyst	% label claimed	
	RIT	LOP
1	99.85	98.62
2	99.72	98.91
3	99.65	99.73
ii) Different days		
Days	% label claim	
	RIT	LOP
1	99.26	99.82
2	98.43	98.71
3	98.51	98.52
Mean	98.74	99.02

Thus, the results obtained by the proposed first order derivative method has determined the percent content of RIT as 99.54±0.42 and LOP as 100.07±0.14 in bulk drug mixture whereas the analysis of marketed tablet estimated the percent of the label claim as RIT- 98.17±0.43 and LOP- 101.6±0.55. The recovery study done by standard addition method has given satisfactory results as RIT -99.05±0.17 and LOP- 99.03±0.21 respectively. Validation of the proposed method was carried out as per ICH guidelines and the results obtained were found to be satisfactory.

CONCLUSION: The first order derivative method proposed for the simultaneous determination of RIT and LOP was found to be accurate and sensitive as the absorptivity of LOP was less and at lower concentrations it was difficult to quantify it.

Hence, the present method is quite good and simple for the estimation of both the drugs in combined tablet dosage form.

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